(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 795 606 A1

(12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
17.09.1997 Bulletin 1997/38

(21) Application number: **95938599.8**

(22) Date of filing: **29.11.1995**

(51) Int. Cl.[6]: **C12N 15/87**, C12N 5/10, C07K 14/78

(86) International application number:
PCT/JP95/02425

(87) International publication number:
WO 96/17073 (06.06.1996 Gazette 1996/26)

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE

(30) Priority: **29.11.1994 JP 317721/94**

(71) Applicant: **TAKARA SHUZO CO. LTD.**
Fushimi-ku Kyoto 612 (JP)

(72) Inventors:
- **HASHINO, Kimikazu**
  Osaka 569 (JP)
- **MATSUSHITA, Hideyuki,**
  Hamoparesu-Kusatsu 401
  Kusatsu-shi, Shiga 525 (JP)
- **KATO, Ikunoshin**
  Kyoto 611 (JP)

(74) Representative: **Vossius, Volker, Dr. et al**
Dr. Volker Vossius,
Patentanwaltskanzlei - Rechtsanwaltskanzlei,
Holbeinstrasse 5
81679 München (DE)

(54) PROCESS FOR PRODUCING TRANSFORMED CELL

(57) A process for producing transformed cells by introducing foreign genes into target cells through piercing, which comprises the step of culturing the target cells having the foreign genes injected thereinto in the presence of a cell adhesion-active substance; and a kit for producing transformed cells suitable for use in the above method and containing as the essential ingredients the cells to be transformed with foreing genes by this method and a cell adhesion-active substance.

EP 0 795 606 A1

Printed by Rank Xerox (UK) Business Services
2.14.14/3.4

Description

TECHNICAL FIELD

The present invention relates to a method for production of transfected cells, more particularly, a method which makes possible to effectively transfer a foreign gene into target cells in the field such as cell technology, genetic engineering, developmental engineering and the like.

BACKGROUND ART

As a method for transferring a foreign gene into target cells, there are known a calcium phosphate method, a DEAE-dextran method, a liposome method, an electroporation method, a microinjection method, a particle gun method and the like. All of these methods have advantages and disadvantages in respect of manipulation procedures, efficacy, damage on cells and the like. Among these methods, a perforation method such as an electroporation method, a microinjection method, a particle gun method and the like can easily handle cells without using special reagents and have good transfer efficacy. However, damage of cells by perforation can not be avoided.

The object of the present invention is to provide a method for improving the transfer efficacy when a foreign gene is transferred into target cells by a perforation method to produce transfected cells.

SUMMARY OF THE INVENTION

The first aspect of the present invention relates to a method for production of transfected cells and is characterized in that said aspect includes a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance, in a method for production of a transfected cell using a perforation method.

The second aspect of the present invention relates to gene-transferred cells which are produced by the method of the present invention.

The third aspect of the present invention relates to a kit for production of transfected cells, which is used for a method for production of transfected cells according to the first aspect of the present invention and is characterized in that said aspect contains a cell-adhering active substance.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is characterized in that, after a foreign gene is transferred into target cells using a perforation method, the cell is cultured in the presence of a substance having the cell adhesive activity.

As used herein, the perforation method means a method for injection of a gene by perforating a cell wall, including an electroporation method, a microinjection method, a particle gun method and the like. The electroporation method is as described in, for example, Tanpakushitsu, Kakusan, Koso, volume 31, page 1591-1603 (1986). The microinjection method is as described in, for example, Cell, volume 22, page 479-488 (1980). The particle gun method is as described in, for example, Technique, volume 3, page 3-16 (1991). These methods include the known methods used for transferring a gene into cells.

For cells used in these perforation methods, for example, animal cells may be prepared according to a known method ["Shin-Seikagaku Jikkenkoza 18, Saibobaiyogijyutsu", 1st edition (1990), edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin] or cultured animal cells may be used.

As used herein, a cell-adhering active substance refers to a substance having the cell-adhering activity, that is, the activity to make target cells adhere to a cell, or to an extracellular matrix which is a substance filling a space between cells in the tissue, or to a material such as plastic, glass and the like. In the present invention, any substances having the activity can be used as long as they give no adverse effects on transfection of target cells. Such the activity is to fix cells, for example, to a culture wear covered with a cell-adhering active substance while maintaining the cell in its form, or in the spreaded form, that is, in the changed form after the cell has been spreaded in one or more directions.

Attachment between the cell-adhering active substance and the target cell can be assayed using a conventional method. The method includes, for example, a method described in Nature, 352: 438-441 (1991). Briefly, the cell-adhering active substance covers a plastic dish and a population of cells to be assayed is put into medium, allowing to stand for 30 minutes to 2 hours. After this incubation period, non-adhered cells are recovered, counted and assayed for viability. Cells adhered to the cell-adhering active substance are recovered using trypsin or a cell dissociation buffer (for example, Gibco), counted and tested for viability. Then, a proportion of adhered cells is calculated and compared with standard or standard control such as a plastic dish covered with bovine serum albumin (BSA). A combination of cell-adhering active substance/cell can be determined by substantial adhesion of the target cell with the cell-adhering active substance assayed. In addition, the cell-spreading activity can be determined by observing under a microscope a

change in the form before adhered cells are dissociated using trypsin or a cell dissociation buffer, in the above procedures.

Examples of the cell-adhering active substance include, for example, a cell-adhering active polypeptide or a functional equivalent thereof and a cell-adhesive synthetic polymer.

Examples of the polypeptide, used in the present invention, having the cell-adhering activity include a cell-adhering active polypeptide such as invasin, polylysine and the like other than that derived from extracellular matrix, for example, a polypeptide showing the cell-spreading activity described in JP-A 2-311498, for example, components of an extracellular matrix such as fibronectin, laminin, collagen, vitronectin, osteopontin, thrombospondin, tenasin and the like. The extracellular matrix components can be prepared from a natural or cultured source by the known method [International Journal of Cancer, volume 20, page 1-5 (1977); Journal of Biological Chemistry, volume 254, page 9933-9937, (1979); "Zoku-Seikagaku Jikkenkoza, volume 6, Saibokokkaku no Kozo to Kino (Structure and Function of Cell Skeleton) (last volume), (1st edition) (1986) edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin; Cell Structure and Function, volume 13, page 281-292 (1988); Journal of Biological Chemistry, volume 264, page 18202-18208 (1989); and Journal of Biological Chemistry, volume 260, page 12240-12245 (1985)]. The cell-adhering active polypeptide may be substantially purified extracellular matrices exhibiting the cell-adhering activity, substantially purified extracellular matrix fragments or a mixture thereof. More particularly, proteins and polypeptides having the cell-adhering activity or the cell-spreading activity, or a functional equivalent thereof may be used.

As these cell-adhering active polypeptides, substantially purified natural polypeptides, polypeptides from enzymological or chemical degradation of the natural polypeptides, or the similar polypeptides made by genetic engineering may be used. Further, materials obtained by altering these polypeptides without impairing the function, that is, the cell-adhering activity or the cell-spreading activity may be used. In the present invention, even when the amino acid sequence of a polypeptide from natural origin has deletion, substitution, addition and/or insertion of an amino acid, as long as the polypeptide has the desired cell-adhering activity or the cell-spreading activity, it is referred to as a functional equivalent of a polypeptide having the natural amino acid sequence. That is, it is known that naturally occurring proteins include proteins of which amino acid sequences have mutation such as deletion, insertion, addition, substitution and the like of an amino acid due to modification reaction in the living body after production or during purification, in addition to proteins having a change in the amino acid sequence due to polymorphism or mutation of genes encoding those naturally occurring proteins and that, regardless of these, there are proteins exhibiting the physiological and biological activity substantially equivalent to that of proteins having no mutation. Like this, even when there is a structural difference between polypeptides, as long as they share the common main functions, they are called polypeptides having the functionally equivalent activity.

This is also true where the above mutations are artificially introduced into the amino acid sequence of proteins. In this case, more variety of mutants may be made. As long as these mutants exhibit the physiological activity substantially equivalent to that of proteins having no mutation, they are interpreted to be a polypeptide having the functionally equivalent activity.

For example, in many cases, a methionine residue present at a N-terminal of a protein expressed in Escherichia coli is said to be removed by an action of methionine aminopeptidase, thus, generating both proteins having a methionine residue or those having no methionine residue depending upon the kind of proteins. However, whether or not a protein has a methionine residue dose not affect on the protein activity in many cases. In addition, it is known that a polypeptide where a certain cysteine residue is substituted with a serine residue in the amino acid sequence of human interleukin-2 (IL-2) retains the interleukin-2 activity [Science, volume 224, page 1431 (1984)].

Further, upon production of proteins by genetic engineering, it is frequently conducted that the proteins are expressed as a fused protein. For example, in order to increase an amount of an expressed protein of interest, it is conducted that the protein is expressed by adding a N-terminal peptide chain derived from other protein to a N-terminal of the protein of interest, or adding a suitable peptide chain to a N-terminal or a C-terminal of the protein of interest to facilitate purification of the protein of interest by using a carrier having the affinity to the added peptide chain.

In this respect, the related biotechnological techniques have progressed and, as the result, deletion, substitution, addition or other modification of an amino acid in a functional area of a subject can be routinely carried out. Then, the resulting amino acid sequence may be routinely screened for the desired cell-adhering activity or the cell-spreading activity according to the above method.

Polypeptides having the cell-adhering activity may be an artificial polypeptide containing, in the molecule, the amino acid sequence necessary for the cell-adhering activity, for example, the amino acid sequence may be selected from the amino acid sequence represented by SEQ ID: No. 1 (RGDS), the amino acid sequence represented by SEQ ID: No. 2 (CS1) and the amino acid sequence represented by SEQ ID: No. 6 (central sequence of laminin, YIGSR). These polypeptides can be prepared in a large amount by a genetic engineering method or chemical synthesis method and may be used as a purified polypeptide.

Examples of the artificial polypeptide having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 include a polypeptide represented by SEQ ID: NO. 7 described in JP-A 1-180900. The polypeptide can be prepared using Escherichia coli HB101/pTF1409 (FERM BP-1939) according to a method described in JP-A 1-180900. In

addition polypeptides represented by respective sequence ID numbers in the sequence list shown in Table 1 below can be prepared according to a genetic engineering method described in each specification.

In addition, a plasmid HB101/pCHV90 contained in Escherichia coli HB101/pCHV90 in Table 1 can be prepared using Escherichia coli HB101/pHD101 (FERM BP-2264) and Escherichia coli JM109/pTF7021 (FERM BP-1941) according to a method described in JP-A 5-271291.

Table 1

Laid Open publication	SEQ ID: No.	Living bacterium (Escherichia coli)	Accession No.
JP-A 1-206998	8	JM109/pTF7021	FERM BP-1941
JP-A 1-261398	9	HB101/pTF1801	FERM P-9948
JP-A 2-97397	3	JM109/pTF7221	FERM BP-1915
JP-A 2-152990	10	JM109/pTFB800	FERM BP-2126
JP-A 2-311498	11	HB101/pCH101	FERM BP-2799
JP-A 3-59000	12	JM109/pCF406	FERM P-10837
JP-A 3-232898	13	HB101/pCE102	FERM P-11226
JP-A 4-54199	14	JM109/pTF7520 +VN-IN.TAA	FERM P-11526
	15	JM109/pTF7520 +Col^{X1}	FERM P-11527
JP-A 5-271291	16	HB101/pCHV179	FERM P-12183
	17	HB101/pCHV90	-
	18	HB101/pCHV89	FERM P-182
JP-A 5-97698	19	JM109/pTF7520ColV	FERM BP-5277
JP-A 5-178897	20	JM109/pYMH-CF・A	FERM BP-5278

Alternatively, artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 can be chemically synthesized. For example, PolyRGDS described in JP-A 3-173828 can be synthesized and used.

Examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 include a polypeptide represented by SEQ ID: No. 4 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pHD102 (FERM P-10721) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 2 may be chemically synthesized according to a method described in JP-A 3-284700.

Further, examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 and the amino acid sequence represented by SEQ ID: No. 3 include a polypeptide represented by SEQ ID: No. 21 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCH102 (FERM BP-2800) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 5 described in JP-A 3-284700 is a polypeptide containing, in the molecule, the amino acid sequences of SEQ ID: No. 1 and 2 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCS25 (FERM P-11339) according to a method described in JP-A 3-284700.

As described above, examples of the polypeptides used in the present invention are cell-adhering active polypeptides containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2. As the polypeptide, a polypeptide obtained by covalently binding a polypeptide derived from a cell adhesion domain of human fibronectin ["Fibronectin", page 47-121 (1989), edited by Mosher, D.F., published by Academic Press] with a CS1 polypeptide derived from the same (ibid), a polypeptide derived from a heparin binding domain (ibid) containing a CS1 polypeptide, or a polypeptide derived from cell adhesion can be used, and they can be made by genetic engineering, respectively. For example, respective necessary regions are taken out from a vector containing a DNA encoding a cell adhesion domain-derived polypeptide, a vector containing a DNA encoding a CS1 polypeptide, and a vector containing a DNA encoding a heparin binding domain-derived peptide containing a CS1 polypeptide, respectively, and they can be used alone or in combination thereof to make a vector expressing a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.

When a polypeptide where a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 are covalently bound is made, a covalent bonding between polypeptides may be a direct bonding or an indirect bonding, for example, an indirect bonding via a spacer. A spacer is an insertion sequence for adjusting an intermolecular distance in each region. As the spacer, an arbitral peptide chain can be used, for example, a sequence upstream of a CS1 region in fibronectin molecule. The spacer sequence can be easily introduced therein by genetic engineering.

The cell-adhesive synthetic polymers include the known poly-N-p-vinylbenzyl-D-lactoneamide (PVLA).

In the present invention, the target cell include, but being not limited to, hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte, cancer cell and the like.

Examples of the foreign gene include, but being not limited to, nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes). In the present invention, the foreign gene may be inserted into a vector.

Examples of the vector are retrovirus vector, adenovirus vector, vacciniavirus vector, herpesvirus vector and the like.

According to the present invention, a target cell into which a foreign gene has been transferred by a perforation method according to a conventional method can be cultured in the presence of a cell-adhering active substance to effectively obtain transfected cells with a transferred gene. A cell culture method may be selected from the known methods depending upon a cell used. For example, when cell culturing is performed in the presence of a cell-adhering active polypeptide, 250 to 2000 μg/ml of the cell-adhering active polypeptide may be used in a culture medium to culture it according to a conventional method.

Particularly, culturing is preferably carried out using a culture wear covered with a cell-adhering active substance. The culture wear refers to any wear normally used for cell culture, for example, a culture dish, a culture wear using a microcarrier, and a culture wear using fibrous hollow fibers. The culture wear may be covered with the substance by coating or spraying. For example, the culture wear may be easily covered with the cell-adhering active substance. The culture wear may be easily covered with the polypeptide by dissolving it in a suitable solution such as a phosphate buffered saline (PBS), adding the solution to the culture wear and allowing to stand for a suitable period of time. An amount of the polypeptide with which the culture wear is covered may be selected from a range of 50 to 1000 pmol/cm^2, suitably 150 to 600 pmol/cm^2.

Transfected cells which have been cultured in the presence of the cell-adhering active substance can be obtained from a culture according to a conventional method. Thus, transfected cells can be produced effectively.

The resulting transfected cells are useful for production of useful substances by cells using gene recombination techniques, exploitation of disease models, gene therapy and the like. Thus, transfected cells can be effectively produced according to the present invention.

In addition, the present invention can be simply carried out by using a kit containing a cell-adhering active substance. The cell-adhering active substance to be contained in the kit may be in a form of solutions or lyophilized powders. The kit may contain a buffer for dissolving or diluting the cell-adhering active substance, a cell culture medium, a cell culture wear and the like. For example, a transfected cell can be simply produced by preparing a kit combining polypeptides, PBS for diluting the polypeptide, a cell culture wear and the like which are used for the method of the present invention. A reagent contained in the kit may be liquid or lyophilized.

A perforation method in the present invention can be used by appropriately selecting from an electroporation method, a microinjection method, a particle gun method and the like depending upon the purpose.

The present invention is illustrated by Examples below but is not limited to them.

Example 1

1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C - CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μM, respectively, which were steriled using a 0.22 μm filter (Millex-GV, Millipore).

Each 1 ml/well of these solutions was added to a 24-well polystyrene culture dish (manufactured by Corning), respectively, to coat the dish at 4 °C overnight. These dishes were rinsed with a 500 μl/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cells

Two culture dishes (diameter: 100 mm) of human epidermoid cancer cell A-431 which had been cultured in a Dul-

becco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 10 ml of PBS, a 3/10 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells were suspended again in 10 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 μg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 μF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of which were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO_2 gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added thereto, followed by culturing at 37 °C in the presence of 5% CO_2 gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT.

The results thereof are shown in Fig. 1. That is, Fig. 1 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 1, an amount of expressed CAT in the culture dish in the C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 2

1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C • CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μM, respectively, which were steriled using a 0.22 μm filter (Millex-GV, Millipore). 1 ml/well of these solutions were added to a 24-well polystyrene culture dish (manufactured by Corning) to coat the dish at 4 °C overnight, respectively. These dishes were rinsed with 500 μl/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cell

Two culture dishes (diameter: 100 mm) of African green monkey kidney cell COS-7 which had been cultured in a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 12 ml of PBS, a 5/6 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells

were suspended in 6 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 μg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 μF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of the cells were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO_2 gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added, followed by culturing at 37 °C in the presence of 5% CO_2 gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT. The results thereof are shown in Fig. 2. That is, Fig. 2 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 2, an amount of expressed CAT in the culture dish in the above C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 3

Preparation of kit

A kit for production of gene-transfered cells was made from C274, H296, C • CS1, PBS and a culturing dish as shown in Table 2 below. Reagents A, B and C were prepared so that the above polypeptides were adjusted with PBS to indicated concentrations shown in the Table. Other components were used which are described in Example 1. In addition, all of reagents A, B and C and a diluent for reagents were aseptically prepared by pre-filtering with a 0.22 μm sterile filter.

Table 2

Kit for production of transfected cell	
Reagent A • • • 100 μM C274	150 μl
Reagent B • • • 100 μM H296	150 μl
Reagent C • • • 100 μM C • CS1	150 μl
Diluent for reagents • • • PBS	45 ml
24-well polystyrene culture dish	3

As described above, the present invention can overcome the problems of the previous methods for gene transfer into cells and provide a method, for production of transfected cells, having improved efficacy of gene transfer into target cells. The present invention can also provide a kit, for production of transfected cells, which are used for the method.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into human epidermoid cancer cell A-431.

Fig. 2 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into African green monkey kidney cell COS-7.

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Takara Shuzo Co., Ltd.
(B) STREET: 609, Takenaka-cho, Fushimi-ku
(C) CITY: Kyoto-shi, Kyoto
(E) COUNTRY: Japan
(F) ZIP: 612

(ii) TITLE OF INVENTION: Method for production of transfected cells

(iii) NUMBER OF SEQUENCES: 21

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: MS-DOS (Version 5.0)
(D) SOFTWARE: Microsoft Word

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: EP 95 93 8599.8
(B) FILING DATE:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/JP95/02425
(B) FILING DATE: 29. November 1995

(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Arg Gly Asp Ser
1

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His
5 10 15
Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
20 25

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 274
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1               5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
            20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
        35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
    50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
            95                  100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
        110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
    125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
            185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
        200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
    215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                260                 265                 270
Thr Glu Ile Asp
```

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 296
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro
 1               5                  10                  15
```

```
Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr
                20                  25                  30
Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
                35                  40                  45
Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser
                50                  55                  60
Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu
                65                  70                  75
Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr
                80                  85                  90
Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala
                95                  100                 105
Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr
                110                 115                 120
Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr
                125                 130                 135
Pro Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile
                140                 145                 150
Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr
                155                 160                 165
Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser
                170                 175                 180
Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr
                185                 190                 195
Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile
                200                 205                 210
Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg
                215                 220                 225
Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile
                230                 235                 240
Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala
                245                 250                 255
Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys
                260                 265                 270
Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu
                275                 280                 285
His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
                290                 295
```

(2) INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 302
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                65                  70                  75
```

```
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
               110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
               125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
               140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
               155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
               170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
               185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
               200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
               215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
               230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
               245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
               260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Asp Glu Leu Pro Gln Leu Val Thr
               275                 280                 285
Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro
               290                 295                 300
 Ser Thr
```

(2) INFORMATION FOR SEQ ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Tyr Ile Gly Ser Arg
  1               5
```

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 283
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Ala Val Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro
  1               5                  10                  15
Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu
               20                  25                  30
Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp
```

```
                35                  40                  45
Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu
                50                  55                  60
Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser
                65                  70                  75
Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys
                80                  85                  90
Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr
                95                 100                 105
Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile
               110                 115                 120
Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg
               125                 130                 135
Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu
               140                 145                 150
Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala
               155                 160                 165
Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
               170                 175                 180
Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr
               185                 190                 195
Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val
               200                 205                 210
Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro
               215                 220                 225
Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile
               230                 235                 240
Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala
               245                 250                 255
Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser
               260                 265                 270
Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Gln Met
               275                 280
```

(2) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 279
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
```

```
                    110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                    125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                    140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                    155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                    170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                    185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                    200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                    215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                    230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                    245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                    260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Gln Met
                    275
```

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 474
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Ala Val Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro
  1                   5                  10                  15
Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu
                    20                  25                  30
Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp
                    35                  40                  45
Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu
                    50                  55                  60
Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser
                    65                  70                  75
Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys
                    80                  85                  90
Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr
                    95                 100                 105
Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile
                   110                 115                 120
Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg
                   125                 130                 135
Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu
                   140                 145                 150
Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala
                   155                 160                 165
Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
                   170                 175                 180
Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr
```

```
                185                 190                 195
Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val
                200                 205                 210
Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro
                215                 220                 225
Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile
                230                 235                 240
Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala
                245                 250                 255
Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser
                260                 265                 270
Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Gln Asn Glu Gly
                275                 280                 285
Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val
                290                 295                 300
Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser
                305                 310                 315
Gly Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His
                320                 325                 330
Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn
                335                 340                 345
Tyr Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln
                350                 355                 360
Met Met Ser Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys
                365                 370                 375
Cys Asp Pro His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr
                380                 385                 390
His Val Gly Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys
                395                 400                 405
Ser Cys Thr Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn
                410                 415                 420
Cys Arg Arg Pro Gly Gly Glu Pro Ser Pro Glu Gly Thr Thr Gly
                425                 430                 435
Gln Ser Tyr Asn Gln Tyr Ser Gln Arg Tyr His Gln Arg Thr Asn
                440                 445                 450
Thr Asn Val Asn Cys Pro Ile Glu Cys Phe Met Pro Leu Asp Val
                455                 460                 465
Gln Ala Asp Arg Glu Asp Ser Arg Glu
                470
```

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 385
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Ala Pro Ile Val Asn Lys Val Val Thr Pro Leu Ser Pro Pro Thr
  1                 5                  10                  15
Asn Leu His Leu Glu Ala Asn Pro Asp Thr Gly Val Leu Thr Val
                 20                  25                  30
Ser Trp Glu Arg Ser Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile
                 35                  40                  45
Thr Thr Thr Pro Thr Asn Gly Gln Gln Gly Asn Ser Leu Glu Glu
                 50                  55                  60
Val Val His Ala Asp Gln Ser Ser Cys Thr Phe Asp Asn Leu Ser
```

```
                    65                  70                  75
Pro Gly Leu Glu Tyr Asn Val Ser Val Tyr Thr Val Lys Asp Asp
                    80                  85                  90
Lys Glu Ser Val Pro Ile Ser Asp Thr Ile Ile Pro Ala Val Pro
                    95                 100                 105
Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met
                   110                 115                 120
Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe
                   125                 130                 135
Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu
                   140                 145                 150
Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu
                   155                 160                 165
Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu
                   170                 175                 180
Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu
                   185                 190                 195
Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser
                   200                 205                 210
Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr
                   215                 220                 225
Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu
                   230                 235                 240
Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu
                   245                 250                 255
Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly
                   260                 265                 270
Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser
                   275                 280                 285
Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser
                   290                 295                 300
Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr
                   305                 310                 315
Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu
                   320                 325                 330
Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu
                   335                 340                 345
Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly
                   350                 355                 360
Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr
                   365                 370                 375
Arg Thr Glu Ile Asp Lys Pro Ser Gln Met
                   380                 385
```

(2) INFORMATION FOR SEQ ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 549
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                   20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
```

```
                    35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                    50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                    65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                    80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                    95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                   110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                   125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                   140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                   155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                   170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                   185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                   200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                   215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                   230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                   245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                   260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Pro Ala Pro Thr Asp
                   275                 280                 285
Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp
                   290                 295                 300
Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr
                   305                 310                 315
Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro
                   320                 325                 330
Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys
                   335                 340                 345
Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg
                   350                 355                 360
Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro
                   365                 370                 375
Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile
                   380                 385                 390
Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp
                   395                 400                 405
Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys
                   410                 415                 420
Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr
                   425                 430                 435
Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser
                   440                 445                 450
Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser
                   455                 460                 465
Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser
```

```
                470                 475                 480
Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr
                485                 490                 495
Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg
                500                 505                 510
Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr
                515                 520                 525
Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser
                530                 535                 540
Glu Pro Leu Ile Gly Arg Lys Lys Thr
                545
```

(2) INFORMATION FOR SEQ ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 422
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                 20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                 35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                 50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                 65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                 80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                 95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Asn Glu Gly Leu Asn Gln
```

```
                    275                 280                 285
Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val Ser His Tyr
                    290                 295                 300
Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser Gly Phe Lys
                    305                 310                 315
Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His Phe Arg Cys
                    320                 325                 330
Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr Lys Ile
                    335                 340                 345
Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met Ser
                    350                 355                 360
Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro
                    365                 370                 375
His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly
                    380                 385                 390
Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr
                    395                 400                 405
Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg
                    410                 415                 420
Pro Gly
```

(2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 332
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                   5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                     20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                     35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                     50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                     65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                     80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                     95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                    110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                    125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                    140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                    155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                    170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                    185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                    200                 205                 210
```

```
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Asn Ser Asp Ser Glu Cys
                275                 280                 285
Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp Gly Val Cys Met
                290                 295                 300
Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val Val Gly
                305                 310                 315
Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu
                320                 325                 330
Leu Arg
```

(2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 341
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                 20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                 35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                 50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                 65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                 80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                 95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
```

```
                    230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                    245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                    260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Gly Ile Tyr Ile Ser Gly Met
                    275                 280                 285
Ala Pro Arg Pro Ser Leu Thr Lys Lys Gln Arg Phe Arg His Arg
                    290                 295                 300
Asn Arg Lys Gly Tyr Arg Ser Gln Arg Gly His Ser Arg Gly Arg
                    305                 310                 315
Asn Gln Asn Ser Arg Arg Pro Ser Arg Ala Met Trp Leu Ser Leu
                    320                 325                 330
Phe Ser Ser Lys Asn Ser Ser Ser Val Pro Ala
                    335                 340
```

(2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 446
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                   20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                   35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                   50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                   65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                   80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                   95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                  110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                  125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                  140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                  155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                  170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                  185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                  200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                  215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                  230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
```

```
                245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Val Pro Gly Phe Lys Gly Asp
                275                 280                 285
Met Gly Leu Lys Gly Asp Arg Gly Glu Val Gly Gln Ile Gly Pro
                290                 295                 300
Arg Gly Xxx Asp Gly Pro Glu Gly Pro Lys Gly Arg Ala Gly Pro
                305                 310                 315
Thr Gly Asp Pro Gly Pro Ser Gly Gln Ala Gly Glu Lys Gly Lys
                320                 325                 330
Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly Pro
                335                 340                 345
Lys Gly Ser Thr Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly Glu
                350                 355                 360
Lys Gly Ala Arg Gly Val Ala Gly Lys Pro Gly Pro Arg Gly Gln
                365                 370                 375
Arg Gly Pro Thr Gly Pro Arg Gly Ser Arg Gly Ala Arg Gly Pro
                380                 385                 390
Thr Gly Lys Pro Gly Pro Lys Gly Thr Ser Gly Gly Asp Gly Pro
                395                 400                 405
Pro Gly Pro Pro Gly Glu Arg Gly Pro Gln Gly Pro Gln Gly Pro
                410                 415                 420
Val Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly Arg
                425                 430                 435
Met Gly Cys Pro Gly His Pro Gly Gln Arg Gly
                440                 445
```

(2) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 457
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                 20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                 35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                 50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                 65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                 80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                 95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
```

```
                155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Asn Val Ser Pro Pro Arg Arg
                275                 280                 285
Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp
                290                 295                 300
Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val
                305                 310                 315
Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp
                320                 325                 330
Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr
                335                 340                 345
Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro
                350                 355                 360
Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu
                365                 370                 375
Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln
                380                 385                 390
Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys
                395                 400                 405
Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly
                410                 415                 420
Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr
                425                 430                 435
Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro
                440                 445                 450
Leu Ile Gly Arg Lys Lys Thr
                455
```

(2) INFORMATION FOR SEQ ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 368
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                 20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                 35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
```

```
                    50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                    65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                    80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                    95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                   110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                   125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                   140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                   155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                   170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                   185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                   200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                   215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                   230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                   245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                   260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Asp Ala Pro Ser Asn
                   275                 280                 285
Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp
                   290                 295                 300
Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu
                   305                 310                 315
Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro
                   320                 325                 330
Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu
                   335                 340                 345
Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu
                   350                 355                 360
Pro Leu Ile Gly Arg Lys Lys Thr
                   365
```

(2) INFORMATION FOR SEQ ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 367
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1                 5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                   20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
```

```
                    35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                    50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                    65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                    80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                    95                  100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                    110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                    125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                    140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                    155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                    170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                    185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                    200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                    215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                    230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                    245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                    260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Asn Val Ser Pro Pro Arg Arg
                    275                 280                 285
Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp
                    290                 295                 300
Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val
                    305                 310                 315
Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp
                    320                 325                 330
Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr
                    335                 340                 345
Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro
                    350                 355                 360
Val Val Ile Asp Ala Ser Thr
                    365
```

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 464
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1               5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
```

```
                    20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
            50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
        65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
    80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                95                  100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
            110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
        125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
    140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
            170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
        185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
    200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
            230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
        245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
    260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Gly Ile Arg Gly Leu Lys Gly
275                 280                 285
Thr Lys Gly Glu Lys Gly Glu Asp Gly Phe Pro Gly Phe Lys Gly
            290                 295                 300
Asp Met Gly Ile Lys Gly Asp Arg Gly Glu Ile Gly Pro Pro Gly
        305                 310                 315
Pro Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Arg Gly Gly
    320                 325                 330
Pro Asn Gly Asp Pro Gly Pro Leu Gly Pro Pro Gly Glu Lys Gly
335                 340                 345
Lys Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly
            350                 355                 360
Pro Lys Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly
        365                 370                 375
Glu Lys Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gly
    380                 385                 390
Gln Arg Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly
395                 400                 405
Ile Thr Gly Lys Pro Gly Pro Lys Gly Asn Ser Gly Gly Asp Gly
            410                 415                 420
Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gly
        425                 430                 435
Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly
    440                 445                 450
Lys Asp Gly Leu Pro Gly His Pro Gly Gln Arg Gly Glu Thr
```

455 460

(2) INFORMATION FOR SEQ ID NO: 20:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 432
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
 1               5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
            20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
        35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
    50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
            80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
        95                  100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
    110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
            140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
        155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
    170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
            200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
        215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
    230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
            260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Ala Gly Ser Ile Thr Thr
        275                 280                 285
Leu Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro
    290                 295                 300
Gly His Phe Lys Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly
305                 310                 315
Phe Phe Leu Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg
            320                 325                 330
Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu
        335                 340                 345
Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu
```

```
                    350                 355                 360
Ala Met Lys Glu Asp Gly Arg Leu Leu Ala Ser Lys Cys Val Thr
                    365                 370                 375
Asp Glu Cys Phe Phe Phe Glu Arg Leu Glu Ser Asn Asn Tyr Asn
                    380                 385                 390
Thr Tyr Arg Ser Arg Lys Tyr Thr Ser Trp Tyr Val Ala Leu Lys
                    395                 400                 405
Arg Thr Gly Gln Tyr Lys Leu Gly Ser Lys Thr Gly Pro Gly Gln
                    410                 415                 420
Lys Ala Ile Leu Phe Leu Pro Met Ser Ala Lys Ser
                    425                 430
```

(2) INFORMATION FOR SEQ ID NO: 21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 574
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

```
Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
  1               5                  10                  15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                 20                  25                  30
Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                 35                  40                  45
Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                 50                  55                  60
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                 65                  70                  75
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                 80                  85                  90
Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                 95                 100                 105
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                110                 115                 120
Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                125                 130                 135
Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                140                 145                 150
Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                155                 160                 165
Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                170                 175                 180
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                185                 190                 195
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                200                 205                 210
Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                215                 220                 225
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                230                 235                 240
Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                245                 250                 255
Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                260                 265                 270
Thr Glu Ile Asp Lys Pro Ser Met Ala Ile Pro Ala Pro Thr Asp
```

```
                    275                 280                 285
Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp
                290                 295                 300
Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr
                305                 310                 315
Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro
                320                 325                 330
Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys
                335                 340                 345
Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg
                350                 355                 360
Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro
                365                 370                 375
Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile
                380                 385                 390
Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp
                395                 400                 405
Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys
                410                 415                 420
Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr
                425                 430                 435
Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser
                440                 445                 450
Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser
                455                 460                 465
Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser
                470                 475                 480
Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr
                485                 490                 495
Glu Lys Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg
                500                 505                 510
Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr
                515                 520                 525
Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser
                530                 535                 540
Glu Pro Leu Ile Gly Arg Lys Lys Thr Asp Glu Leu Pro Gln Leu
                545                 550                 555
Val Thr Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp
                560                 565                 570
Val Pro Ser Thr
```

Claims

1. In a method for production of transfected cells by transferring a foreign gene into target cells using a perforation method, said method for production of cells transfected with a foreign gene which comprises a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance.

2. The method for production of transfected cells according to claim 1, the culturing step is a step of culturing using a culture wear covered with a cell-adhering active substance.

3. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is a cell-adhering active polypeptide or a functional equivalent of said polypeptide.

4. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is

a cell-adhering and/or cell-spreading active polypeptide.

5. The method for production of transfected cells according to claim 3, wherein the cell-adhering and/or cell-spreading active polypeptide is a polypeptide containing the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.

6. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is selected from polypeptides represented by SEQ ID: Nos. 3, 4 and 5.

7. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is poly-N-p-vinylbenzyl-D-lactoneamide.

8. The method for production of transfected cells according to claim 1, wherein the target cells are selected from hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte and cancer cell.

9. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes).

10. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes) and the nucleic acid is incorporated into the vector.

11. The method for production of transfected cells according to claim 1, wherein the vector is a vector selected from retrovirus vector, adenovirus vector, vacciniavirus vector and herpesvirus vector.

12. The method for production of transfected cells according to claim 1, the perforation method is selected from an electroporation method, a microinjection method and a particle gun method.

13. Transfected cells produced by a method for production of transfected cells according to claim 1.

14. A kit for production of transfected cells with a foreign gene which is used in a method for production of transfected cells according to claim 1, said kit comprises containing a cell-adhering active substance.

Fig. 1

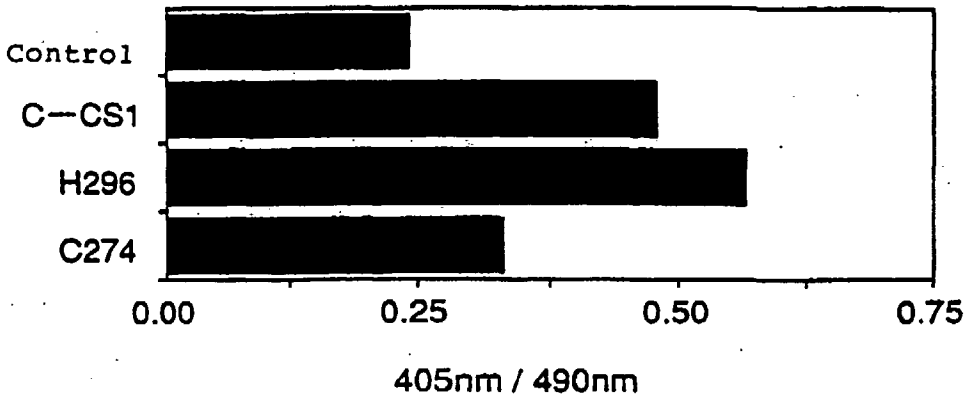
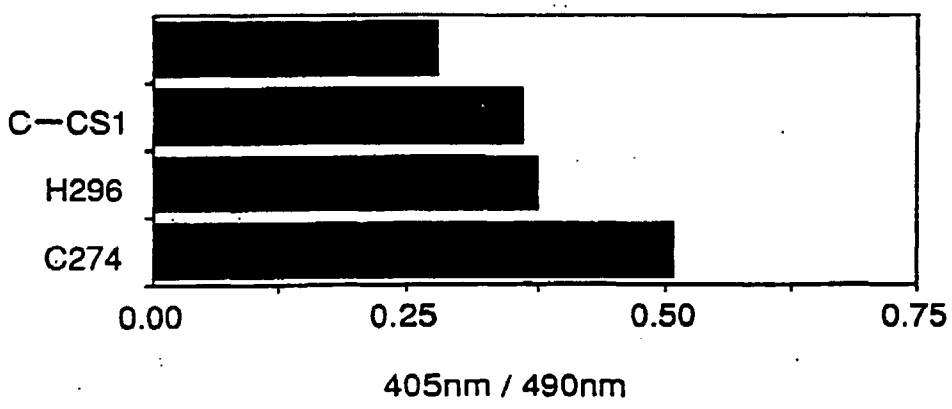

Control
C—CS1
H296
C274
0.00
0.25
0.50
0.75
405nm / 490nm

Fig. 2


C—CS1
H296
C274
0.00
0.25
0.50
0.75
405nm / 490nm

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/02425

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 C12N15/87, C12N5/10, C07K14/78
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 C12N15/87, C12N5/10, C07K14/78

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI, WPI/L, BIOSIS PREVIEWS
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, 4-063597, A (W.R. Grace & Co.), February 28, 1992 (28. 02. 92) & EP, 463508, A & CA, 2044307, A	1 - 14
A	JP, 6-090771, A (Shiseido Co., Ltd.), April 5, 1994 (05. 04. 94)(Family: none)	1 - 14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
March 1, 1996 (01. 03. 96)	March 19, 1996 (19. 03. 96)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)